148 *Burkholderia cepacia* complex isolation and identification in a Turkish CF Unit

P. Yurdakul[1], K. Ergünay[1], E. Yalcin[2], D. Dogru[2], B. Sener[1], N. Cobanoglu[2], U. Ozcelik[2], N. Kiper[2]. [1]*Dept. of Clinical Microbiology,* [2]*Pediatric Pulmonology Unit, Hacettepe Univ. Medical Faculty, Ankara, Turkey*

Introduction: Isolation of *B. cepacia* complex (Bcc) in sputum from CF patients carries a significant medical importance. Rapid and precise identification is essential to evaluate risks in terms of prognosis and epidemicity. This study was performed to evaluate the prevalence and genomovar status of Bcc in our CF population.
Methods: Sputum samples colllected from 92 CF patients were inoculated onto OFBL agar for the isolation of Bcc. Phoenix© Identification System was used for phenotypic analysis. Nucleic acid purification from sputum was performed using High Pure PCR Template Kit©. Bcc *rec* A gene was amplified utilizing BCR-1 and BCR-2 primers. Bcc strain KK 7394 (Neqas, UK) was used as reference strain and *P. putida* as negative control. For the validation of PCR, another CF sputum with no pathogenic bacterial growth was spiked with 10^7 cfu/ml reference strain. Restriction fragment length polymorphism (RFLP) was done for the molecular identification of Bcc isolate.
Results: Bcc was isolated from 2 out of 92 samples (2.2%) and phenotypically verified using the automatized system. PCR was shown to detect DNAs from reference strain, spiked sample and one of the sputum samples from which Bcc was isolated (1.1%). *RecA* PCR (+) strain was identified as *B. multivorans* by RFLP.
Conclusion: Although Bcc is an infrequent pathogen in our CF population, special care should be given for its isolation and identification since it has considerable impact on morbidity and mortality in CF. Identification of putative Bcc isolates should be confirmed by molecular methods as misidentification may occur when only phenotypic analysis is considered.

149 Clinical features of *Pseudomonas aeruginosa* (Pa) negative cystic fibrosis (CF) patients with the history of Pa infection

V. Vavrova, J. Bartosova, L. Fila, V. Skalicka, J. Brazova, K. Bohmova, S. Vosahlikova, P. Pohunek, D. Zemkova. *CF Centre, Charles University – 2nd Medical School and University Hospital Motol, Prague, Czech Republic*

In our Prague CF Centre, we have observed an increasing number of Pa negative patients with the history of Pa infection (Pa free patients). Here we studied clinical peculiarities in these patients. Overall 39/209 sputum producers had Pa negative culture for at least 1 year (2.8±1.6). Their age and lung function were compared with groups of Pa negative patients (n=39), Pa intermittent positive patients (n=49), patients with Pa chronic colonization, and *Burkholderia cepacia* complex (Bcc) cases (n=54). Pa free patients were younger than patients with Pa chronic colonization and Bcc infected cases, and had similar age as Pa negative and intermittent positive group. With regards to lung function 56.2% of patients had FEV1 higher than 80% p.v. and 6.3% of patients had FEV1 lower than 40% p.v. Compared to Pa negative patients, they had a tendency towards FEV1 lower than 70% p.v. ($p=0.07$). FEV1 of Pa free patients older than 18 years tends to be similar to FEV1 in Bcc infected cases.
All Pa free patients were PCR negative and most of the Pa free patients with FEV1 lower than 70% p.v. had negative anti-Pa antibodies. Our unfavourable findings were not caused by false negative cultures. We suppose that these results in older Pa free patients could be due to irreversible changes that have developed during Pa infection and/or less vigilant antibiotic treatment after repeatedly negative cultures. Therefore, we consider that early diagnosis of the pathogen and an aggressive treatment of Pa primo-infection are necessary.
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150 Assessment of molecular methods for the rapid identification of *Burkholderia cepacia* complex (Bcc)-positive sputa

A.R. Brown, J.R.W. Govan. *The Centre for Infectious Diseases, University of Edinburgh, UK*

Aims: Early implementation of appropriate antimicrobial therapy and infection control measures is reliant on the rapid and accurate diagnosis of respiratory infections. Whilst culture-based methods with subsequent biochemical analysis and species-specific PCR offer reliable identification, this process may take up to a week when applied to sputa, particularly if bacterial counts are low or isolates atypical. The current segregation policy for CF patients with Bcc infection does not distinguish between genomovars. Accordingly, this study aims to assess non-culture-based methods (PCR and fluorescent in situ hybridization, FISH) for the identification of Bcc-positive sputa within 2–3 hours.
Methods: The study will compare commercially-available FISH reagents (SeaPro Theranostics) with a Bcc-specific in-house PCR assay (targeted against 16S rRNA sequence) for the identification of Bcc organisms within CF sputa.
Results: In a preliminary blind study of 100 CF isolates (including 37 Bcc, 1 non-Bcc *Burkholderia*, 33 Pseudomonads, 10 *Achromobacter xylosoxidans*, 2 *Ralstonia* sp., 1 *Pandoraea* sp.) the Bcc PCR assay exhibited a sensitivity and specificity of 97% (36/37) and 100% (37/37) respectively. The single false-negative, a *B. multivorans* isolate, was positive upon re-testing. A comparable study will be performed to assess the specificity of FISH for the Bcc. Following optimization, the PCR- and FISH-based assays will be applied to CF sputa, and results compared with those obtained by conventional methods. The results of this comparison will be presented.
Conclusions: By assessing rapid molecular methods for the identification of Bcc within CF sputa, this study aims to improve on existing diagnostic methods and enhance the management of Bcc infections in CF patients.

151 Identification of *Burkholderia cepacia* complex using MALDI-TOF mass spectrometry

E. Vanlaere[1], K. Sergeant[2], P. Dawyndt[1], B. Devreese[2], P. Vandamme[1]. [1]*Laboratory for Microbiology, University of Gent, Gent;* [2]*Laboratory of Protein Biochemistry and Protein Engineering, University of Gent, Belgium*

The identification of organisms cultured from respiratory specimens obtained from CF patients is not straightforward and misidentifications occur. We evaluated the identification of *Burkholderia cepacia* complex (Bcc)-like organisms using Matrix Assisted Laser Desorption/Ionisation Time Of Flight mass spectrometry (MALDI-TOF MS) of intact microbial cells. In this technique, laser light vaporizes biomolecules achieving free gas-phase ions that were separated by their mass-to-charge ratios. We investigated 60 Bcc strains and 40 non-Bcc strains representing 20 species that are commonly misidentified as Bcc. Colonies grown for 24 h were suspended in 0.5 ml 0.1% TFA, 2 µl of which was mixed with 2 µl matrix solvent (49 ACN: 49 Isopropanol: 2 0.1% TFA saturated with alpha-cyano). Subsequently 1 µl was spotted for analysis.
We obtained bacterial mass spectra in the m/z range 2–18 kDa. The data were analysed by Bionumerics 4.0 software and subjected to a cluster analysis using Pearson's correlation coefficient. Except for *Burkholderia gladioli* and some *Ralstonia sp.*, all reference species were easily distinguished from Bcc. Most strains belonging to Bcc species grouped in species specific clusters, except for *Burkholderia pyrrocinia* and *Burkholderia anthina* that constitute a single cluster. The same was observed for most of the other taxa examined. In the mass spectra some peaks could be used as biomarkers to distinguish between species or even strains. Moreover, this method is quick, easy to perform and rather cheap.
These data suggest that MALDI-TOF MS of intact cells could be a powerful tool for the rapid identification of Bcc-like organisms recovered from CF patients.